

## THE AMINO ACID COMPOSITIONS OF MAJOR AND MINOR AMPULLATE SILKS OF CERTAIN ORB-WEB-BUILDING SPIDERS (ARANEAE, ARANEIDAE)

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### ABSTRACT

Amino acid compositions are given for forcibly secured major ampullate silk ( $n = 59$  from 4 subfamilies, 8 genera, 11 species) and minor ampullate silk ( $n = 11$ , 3 subfamilies, 6 genera, 8 species) of mature, female, orb-web-building, spiders. The compositions of major ampullate silk samples are neither uniform within species nor when taken from an individual spider, or even when taken from an individual spider during a single forcible silking. Chemical compositions of the two types of silk are correlated to a limited degree with their physico-chemical properties and with the taxonomic position of the spiders.

### INTRODUCTION

The objectives were (1) to determine the amino acids in major and minor ampullate silk fibers (MaAS and MiAS, singular and plural) of orb-web-building spiders, and (2) to look for correlations between their chemical compositions and physical properties and the taxonomic position of the spiders (see Work 1977 and 1981b). During the study, it was discovered that these chemical compositions vary in samples from within each species and even from within an individual spider and the study was expanded to investigate these aspects.

Only eleven papers have reported original chemical analyses of the amino acid compositions of spider silks or progenitive silk polypeptides from the major or minor ampullate gland systems. The possibility of variability of the chemical composition within species or among samples taken from a single spider has been ignored. E. Fischer (1907) made the first analysis of spider silk on samples from *Nephila madagascariensis* (Vinson). Braunitzer and Wolff (1955) analyzed the contents of silk glands and silk from the same species and reported that the forcible silk samples sometimes consisted of four fibers, two about  $6\mu\text{m}$  and two of smaller diameters. These samples thereby contained both MaAS and MiAS. Lucas et al. (1960) semi-quantitatively analyzed the silks of 70 species of Insecta

and six arachnid silks through paper chromatography while quantitative analyses included "reeled" silks of *N. madagascariensis* and cocoon (egg) silk of *Nephila senegalensis* (Walckenaer). Lucas (1964) later analyzed the dragline and cocoon of *Araneus diadematus* (Clerck), but supplied no information on conditions of sample procurement or duplicability of analyses. Fischer and Brander (1960) reported the amino acid analyses of frame fibers, dragline, cocoon, and entire web of *A. diadematus*, *Nuctenea sclopetaria* (Clerck) (= *Araneus undatus* (Oliv.)), and *Araniella cucurbitana* (Clerck) (= *Araneus cucurbitanus* (Clerck)). Their Table 1 gives two sets of data on frame fibers and three on dragline from *A. diadematus*, the data in each set being based on means from three separate analyses. Peakall (1964) pooled three to five samples from the same spider (*A. diadematus*) for each analysis and made six to eight duplicate runs, and indicated that the variability for major components was  $\pm 5\%$ . He recognized each of the fibroins might be composed of "a group of proteins rather than a single protein" but upon electrophoresis of the material from the ampullate "only a single, although somewhat diffuse, peak was found". He further noted that Warwicker (private communication) "considers that each gland produces a single protein". Zemlin (1968) analyzed silks from *Nephila clavipes* (Linnaeus), *Nephilengys cruentata* (Fabricius) (= *Nephila cruentata* (Fabricius)), and an unidentified spider from Paneiras, Brazil. He discussed sources of variability, as functions of sample size, in some detail (loc. cit., p. 63) and reported upon two conditions of hydrolysis for one sample. Andersen (1970) analyzed the contents of the silk producing glands, including the major ampullate secured from an unstated number of *A. diadematus* spiders. Tillinghast (1984) determined the amino acid compositions of the contents of six different glands, including major ampullate, taken by dissection from female *Argiope aurantia* Lucas. Tillinghast and Christenson (1984) made similar analyses on the major ampullate gland contents of female *N. clavipes*. The number of specimens used was not stated in either case.

In a study of the morphology and ultrastructure of the duct of the major ampullate gland of *A. diadematus* (Kovoor and Zylberberg, 1972) it was not suggested that its contents may be bicomponent and the same is the general case for observations made in a comprehensive review of arachnid silks (Kovoor, 1977). However, Hans M. Peters illustrated (see Work 1981a, figs. 25 and 26) that two fibers may emerge from single major ampullate spigots. Duality of the luminal contents of ducts and skin-core structures of fibers from these same gland systems have been described (Work 1984). But physical differences in fibers do not imply that these are based on chemical dissimilarity, as is well known among man-made fibers. Nylon and polyester 'stretch garments' depend upon fibers which are physically non-uniform in cross section. Viscose rayon fibers based on a single polymer may have a skin-core morphology, as has been described and illustrated by Morehead and Sisson (1945), Horio and Kondo (1953), Sisson and Morehead (1953), and Sisson (1960). Kovoor and Zylberberg (1980) reported a dual composition in pyriform silk while Palmer, et al. (1982) described chemically differentiable skin-core in the contents of the four silk ducts of *Antrodiaetus unicolor* (Hentz) and (1985) in the silk from spiders of the genus *Euagrus*. Gosline et al. (1984) have carried duality to a molecular level by suggesting that wetted, supercontracted, major ampullate fibers consist of crystallites (x-ray diffraction definition) in a matrix of amorphous polypeptide

rubber. Where pertinent to this paper, chemical analyses recorded in the above-mentioned papers have been compiled in Table 1. In some cases these data will be found to differ from corresponding data in Kovoov's Table 2 (*loc. cit.*, 1977), because the latter were not converted from the original g/100g results to residues/1000 residues, as is captioned for that table. The results given in Table 1 will be discussed later in this paper.

## MATERIALS AND METHODS

**Sampling.**—Forcible silking is limited to those spiders which are large enough to be adequately manipulated, but some resist and make sampling impossible. Responses for an individual spider appear to be essentially fortuitous, even with spiders which have been successfully silked. The operation may last from a few seconds to as long as about fifteen minutes and MaAS or MiAS, or both may be obtained and, as a practical matter, it is impossible to obtain balanced sets of samples with which to make symmetrical measurements.

Mature female spiders provided samples under constant microscopic (mag. = 10-70X) observation as described by Work and Emerson (1982). This made possible separation of MaAS and MiAS whenever the spider produced them simultaneously. Each sample was examined at higher magnification (250-430X) for freedom from contaminants and subsamples were tested for supercontractability to further confirm the glandular source.

Set 1 samples were taken when it had been mistakenly assumed that the MaAS composition was uniform within species and from individual spiders. Set 2 samples were secured to expand the study and better quantify the early indication. Set 3 samples produced time-progressive subsamples for examination of variability during continuous silkings. Samples of MiAS were obtained whenever they were produced.

**Species.**—Samples were obtained from the following araneid species: *Araneus diadematus* Clerck, *Araneus marmoreus* Clerck, *Eriophora fuliginea* (C. L. Koch), *Eriophora ravilla* (C. L. Koch), *Neoscona hentzii* (Keyserling), *Metazygia wittfeldae* (McCook), all subfamily Araneinae; *Argiope argentata* (Fabricius), *Argiope aurantia* Lucas, both subfamily Argiopinae; *Nephila clavipes* (Linnaeus), *Nephilengys cruentata* (Fabricius), both subfamily Nephilinae; *Leucauge argentea* (Keyserling), subfamily Metinae (Classification according to H. W. Levi, *per. comm.*, 1980-81).

**Instrumentation.**—The first group (Set 1) of samples was analyzed using a Beckman 118 amino acid analyzer. This device was incapable of determining amino acids present in very small amounts, but it is estimated that the total amount of these non-measurable components in any single analysis was not greater than two in a total of 100 residues actually present. A Durrum D-500 instrument, capable of analyzing smaller samples, became available for the second group of samples (Set 2). For the third period of the investigation, analyses (Set 3 samples) were divided between the Durrum and a Waters HPLC analyzer. Unfortunately, neither author was aware when the operation was planned that the latter device is incapable of determining proline. Thus for statistical analysis, the proline values secured by means of the Durrum analyzer on the adjacent sample or bracketing samples, were inserted into the Waters data. In the single case

Table 1.—Published literature on the amino acid composition of spider silk: Amino acid residues/100 total residues, i.e., mole % (converted thereto if otherwise reported by the author). Column 1, Author: 1 = Fischer & Brander 1960; 2 = Lucas et al. 1960; 3 = Lucas 1964; 4 = Peakall 1964; 5 = Zemlin 1968; 6 = Andersen 1970; 7 = Tillinghast 1984; 8 = Tillinghast & Christenson 1984. Column 2, Source of Silk: 1 = Frame; 2 = Dragline; 3 = Pulled; 4 = Scaffold; 5 = Major Ampullate Gland; 6 = Reeled; 7 = Foundation; 8 = Minor Ampullate Gland. Column 3, Taxa: 1 = *Araneus diadematus*; 2 = *Nephila clavipes*; 3 = *Nephilengys cruentata*; 4 = *Nephila madagascariensis*; 5 = *Nuctenea sclopetaria* (= *Araneus undatus* Oliv.); 6 = *Araniella cucurbitana* (= *Araneus cucurbitanus* Clerck); 7 = *Argiope aurantia*. ND = Not determined. TR = Trace. \*\*ILE + LEU.

|   | 1 | 2 | 3 | ASP  | THR  | SER  | GLU   | PRO   | GLY   | ALA   | CYS  | VAL   | ILE     | LEU  | TYR  | PHE  | HIS  | LYS  | ARG  | MET  |
|---|---|---|---|------|------|------|-------|-------|-------|-------|------|-------|---------|------|------|------|------|------|------|------|
| 1 | 1 | 1 | 1 | 1.68 | 1.45 | 5.57 | 10.28 | 14.46 | 27.83 | 28.26 | 0.23 | 1.49  | 1.66    | 2.08 | 2.51 | <.11 | ND   | 1.22 | 1.17 | ND   |
| 1 | 1 | 1 | 1 | 1.70 | 1.65 | 7.67 | 9.82  | 14.39 | 29.75 | 24.95 | 0.20 | 1.27  | 1.74    | 2.22 | 2.43 | ND   | ND   | 1.43 | 1.27 | ND   |
| 1 | 2 | 1 | 1 | 0.69 | 1.14 | 8.41 | 12.46 | 15.55 | 30.36 | 21.05 | 0.13 | 2.04  | 0.79    | 1.27 | 3.72 | ND   | ND   | 1.30 | 0.90 | ND   |
| 1 | 2 | 1 | 1 | 2.35 | 2.07 | 7.99 | 11.76 | 13.46 | 33.05 | 19.61 | <.06 | <1.64 | 0.62    | 0.62 | 3.58 | ND   | ND   | 1.63 | 1.44 | ND   |
| 1 | 2 | 1 | 1 | 1.84 | 1.50 | 7.72 | 13.01 | 14.00 | 31.56 | 20.79 | <.06 | 0.97  | 0.84    | 0.84 | 3.66 | ND   | ND   | 1.79 | 1.30 | ND   |
| 3 | 2 | 1 | 1 | 1.4  | 1.2  | 6.3  | 9.4   | 10.6  | 37.2  | 22.6  | 0.6  | 1.2   | 0.6     | 1.4  | 4.6  | 0.40 | 0.00 | 0.9  | 0.9  | 0.50 |
| 4 | 1 | 1 | 1 | 1.0  | 1.5  | 5.5  | 12.0  | 2.7   | 31.3  | 36.8  | ND   | 1.0   | 1.6     | 1.4  | 1.3  | ND   | ND   | 1.5  | 2.4  | ND   |
| 4 | 3 | 1 | 1 | 1.0  | 1.8  | 5.9  | 12.0  | 2.7   | 31.7  | 36.4  | ND   | 1.7   | 1.3     | 1.6  | 1.0  | ND   | ND   | 1.3  | 1.8  | ND   |
| 4 | 4 | 1 | 1 | 1.0  | 1.4  | 6.0  | 12.0  | 2.0   | 31.7  | 37.2  | ND   | 1.1   | 1.8     | 1.1  | 1.1  | ND   | ND   | 1.3  | 2.1  | ND   |
| 6 | 5 | 1 | 1 | 1.04 | 0.91 | 7.41 | 11.49 | 15.77 | 37.24 | 17.60 | ND   | 1.15  | 0.63    | 1.27 | 3.92 | 0.45 | ND   | 0.54 | 0.57 | ND   |
| 5 | 2 | 2 | 2 | 2.5  | 1.2  | 6.9  | 9.0   | 1.1   | 41.5  | 27.0  | 0.0  | 1.2   | 0.6     | 2.0  | 2.7  | 0.5  | TR   | 0.8  | 2.0  | TR   |
| 8 | 5 | 2 | 1 | 1.9  | 1.0  | 3.0  | 10.1  | 1.7   | 40.3  | 28.4  | —    | 1.5   | 0.6     | 4.5  | 3.1  | 0.5  | 0.02 | 0.8  | 2.0  | 0.3  |
| 5 | 2 | 3 | 1 | 1.3  | 0.7  | 3.0  | 10.0  | 2.6   | 43.3  | 30.4  | 0.0  | 0.1   | 0.5     | 1.8  | 4.0  | 0.04 | <.03 | 0.3  | 1.1  | 0.01 |
| 2 | 6 | 4 | 0 | 0.9  | 0.6  | 4.2  | 11.6  | ND    | 40.6  | 32.1  | ND   | 0.9   | ** 2.90 | **   | 3.2  | 0.6  | ND   | ND   | 2.4  | ND   |
| 1 | 7 | 5 | 1 | 3.1  | 1.2  | 9.3  | 10.2  | 8.5   | 30.5  | 13.1  | <.1  | 5.4   | 3.3     | 6.4  | 2.0  | 3.9  | ND   | 1.3  | 1.8  | ND   |
| 1 | 7 | 6 | 2 | 2.8  | 1.7  | 9.1  | 10.6  | 11.1  | 35.1  | 14.4  | <.1  | 2.6   | 1.6     | 5.5  | 1.7  | 0.8  | ND   | 1.6  | 1.3  | ND   |
| 8 | 5 | 7 | 1 | 1.1  | 0.6  | 4.6  | 12.3  | 10.3  | 37.8  | 22.8  | —    | 1.4   | 0.5     | 1.8  | 3.4  | 1.2  | 0.2  | 0.5  | 1.4  | 0.6  |
| 6 | 8 | 1 | 1 | 1.91 | 1.35 | 5.08 | 1.59  | TR    | 42.77 | 36.75 | ND   | 1.73  | 0.67    | 0.96 | 4.71 | 0.41 | TR   | 0.39 | 1.69 | ND   |
| 7 | 8 | 7 | 2 | 2.8  | 2.0  | 5.2  | 2.2   | 0.9   | 41.2  | 31.3  | —    | 2.3   | 1.0     | 1.8  | 4.8  | 0.9  | 0.3  | 1.0  | 2.2  | 0.4  |



where this was not possible, a mean value of all proline determinations for that species was used. Values of all other amino acids were adjusted to 100 total residues. Data are reported in amino acid residues per 100 total residues (mole %).

**Analytical procedures and variances.**—The procedures used for the analyses were those commonly associated with the three instruments. Because of the high percentage of glycine and alanine, when the Beckman and Waters instruments were used, it was necessary to dilute aliquots from the hydrolysate solutions for their analyses.

The potential sources of analytical variances in the procedures are 'machine (instrument)-operator errors'. The three instrument-operator combinations used in the present study have been used on a daily basis for a variety of protein analyses and have been subjected to normal quality maintenance tests to establish precision, accuracy and duplicability of analyses. Although the instrument-operator error is a function of the amount of amino acid present, it is reasonable to report all results to two decimal places (Tables 2, 3 and 5) for the statistical analyses made by the authors and for readers who may wish to extend them.

As an additional check on MaAS, a toroidal bundle from the sampling mandrel was cut on a diameter and the two halves were separately analyzed (Table 2). Duplicate analyses were made at appropriate intervals in the continuing study when hydrolysate quantities permitted.

## RESULTS

The results obtained in the present study are found in Tables 2 and 3 for MaAS and Table 5 for MiAS. In the first column of these, OBS (OBS = observation) refers to an analysis of a single sample of forcibly taken MaAS or MiAS, with the following exceptions. Table 2 provides the analytical results on the two toroidal bundle samples as mentioned before. Mean values from these appear in OBS #32 of Table 3. In Table 3, OBS #44 was comprised of three primary samples and in Table 5, both OBS #7 and #8, were comprised of two primary samples. In all these cases the primary samples were too small to be analyzed alone.

The classification (by H. W. Levi) of the spiders producing the samples is found in the second to fourth columns: SF = subfamily, G = genus, and SP = species. Subfamilies: 1 = Araneinae; 2 = Argiopinae; 3 = Metinae; 4 = Nephilinae. Genera: 1 = *Araneus*; 2 = *Argiope*; 3 = *Eriophora*; 4 = *Leucauge*; 5 = *Nephila*; 6 = *Nephilengys*; 7 = *Metazygia*; 8 = *Neoscona*. Species: 1 = *diadematus*; 2 = *marmoreus*; 3 = *argentata*; 4 = *aurantia*; 5 = *fuliginea*; 6 = *argentea*; 7 = *clavipes*; 8 = *cruentata*; 9 = *wittfeldae*; 10 = *ravilla*; 11 = *hentzii*.

"Spider" and "sample" indicate the source and numbered identity of the samples in the log book (R.W.W.).

"Group" refers only to Set 3 samples. Each numbered group was secured from a single, continuous, forcible silking, and the alphabetical letters indicate the chronological order in which the individual samples were produced.

Analytical instruments ("ANAL") are identified by manufacturer: B = Beckman (Set 1), D = Durrum (Sets 2 and 3), and W = Waters (Set 3).

The remaining columns present the results of the analyses of major (Table 3) and minor (Table 5) ampullate silk samples, expressed in amino acid residues per 100 total residues (mole %).

## DISCUSSION

It has been mentioned that the simple demonstration of skin-core morphology in organic fibers does not alone connote that the two phases differ in chemical composition. But the very limited study of physical behavior of the skin-core structure in MaAS (Work 1984) indicates that its basis is other than the physical factors of segmental orientation, molecular conformations, or crystallite-amorphous domains (x-ray diffraction defined). A chemical difference is suggested by the ease of fracture between skin and core when the primary fiber is allowed to supercontract and is then wet extended. This possibility is vastly reinforced by the results obtained from amino acid analyses. It must be kept in mind that for an individual sample so analyzed, it was not known whether it was single phase or skin-core, and if the latter, what the ratio of these two phases might be.

**Chemical compositions reported in the literature.**—It is seen in Table 1 that items 1-9 of presumed MaAS and the one analysis (item 10) of its progenitive polypeptide from *A. diadematus* are far from uniform. Although the techniques and equipment used at least 25 years ago could be expected to produce variances larger than those found today, the wide differences seen among the analyses suggest something more fundamental than instrument-operator errors. As already noted, the presumed samples of MaAS (but not the progenitive polypeptide) might have been contaminated with MiAS. To the degree that this might have occurred, both glutamic acid and proline would be expected to be reduced while both glycine and alanine would increase. Examination of the data from samples taken from *A. diadematus* show no uniform trends. It is our conclusion that the variability among items 1-10 was not entirely caused by analytical inconsistencies, contamination by MiAS, or a combination of both. It is suggested that they demonstrate real differences in the chemical compositions of MaAS from *A. diadematus* spiders.

**Variability of chemical composition.**—*Set 1 samples.* Set 1 provided nine samples from four species (Table 3, "ANAL" = B and Group is blank). Of these, there were four samples from *A. aurantia*, and three from *N. clavipes*, and in these two subsets, three samples came from spiders 7917 and two from 7915, respectively. When considerably greater variability was found within species (see next section), than from the two halves of a single sample (Table 2), experimental procedures were examined without finding any source of gross error. All spiders maintained in captivity were fed house flies (*Musca domestica*) raised under controlled conditions in the North Carolina State University Department of Entomology. Statistical analyses were made on results on silk samples from left, right, and both major ampullate spigots, without providing any useful information. It was concluded that the variability of chemical composition was not due to instrument or human error, diet, or differences in output of spigots. Rather, the results strongly suggested that the chemical composition of MaAS is neither constant within species nor from a single spider. The search for causes was directed elsewhere (see Work 1984) and more samples were collected for analyses.





|    |   |   |   |      |        |     |      |      |      |       |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
|----|---|---|---|------|--------|-----|------|------|------|-------|------|-------|-------|------|------|------|------|------|------|------|------|------|------|------|
| 27 | 2 | 2 | 4 | 7917 | 179    | B   | 0.91 | 0.31 | 3.92 | 11.14 | 9.44 | 44.84 | 20.16 | 0.13 | 0.87 | 0.48 | 1.60 | 3.00 | 0.82 | 0.00 | 0.57 | 1.33 | 0.00 | 0.49 |
| 28 | 2 | 2 | 4 | 7917 | 215    | B   | 1.13 | 0.46 | 4.13 | 11.30 | 9.90 | 36.55 | 22.34 | 0.41 | 1.38 | 0.90 | 1.55 | 3.55 | 0.88 | 0.00 | 2.40 | 1.43 | 0.00 | 1.68 |
| 29 | 2 | 2 | 4 | 7917 | 164    | D   | 0.83 | 0.28 | 2.60 | 12.26 | 8.91 | 32.57 | 20.85 | 0.57 | 1.08 | 0.21 | 1.55 | 6.12 | 9.62 | 1.74 | 0.27 | 0.54 | ND   | 0.00 |
| 30 | 2 | 2 | 4 | 7917 | 178    | D   | 0.98 | 0.40 | 3.47 | 11.35 | 9.42 | 43.65 | 20.65 | 0.20 | 1.06 | 0.72 | 1.85 | 2.76 | 1.11 | 0.40 | 0.29 | 1.44 | ND   | 0.27 |
| 31 | 2 | 2 | 4 | 7917 | 200    | D   | 1.03 | 0.33 | 2.82 | 11.39 | 9.11 | 40.40 | 24.75 | 0.19 | 0.91 | 0.39 | 2.03 | 2.64 | 1.40 | 1.32 | 0.37 | 0.93 | ND   | 0.00 |
| 32 | 2 | 2 | 4 | 7929 | 243    | B   | 0.83 | 0.31 | 3.52 | 12.06 | 9.07 | 40.07 | 24.90 | 0.12 | 0.76 | 0.48 | 1.82 | 3.15 | 1.07 | 0.06 | 0.40 | 1.36 | 0.00 | 0.00 |
| 33 | 2 | 2 | 4 | 7929 | 239    | D   | 0.73 | 0.09 | 3.50 | 13.86 | 9.63 | 48.31 | 16.82 | 0.19 | 1.06 | 0.17 | 0.60 | 1.71 | 0.68 | 1.09 | 0.19 | 0.78 | ND   | 0.59 |
| 34 | 2 | 2 | 4 | 7933 | 248    | D   | 0.72 | 0.12 | 2.41 | 12.25 | 8.12 | 48.65 | 19.36 | 0.13 | 0.98 | 0.27 | 1.29 | 1.53 | 1.19 | 0.71 | 0.37 | 1.46 | ND   | 0.43 |
| 35 | 2 | 2 | 4 | 8208 | 412    | 6A  | 0.84 | 0.15 | 3.86 | 13.11 | 8.72 | 42.42 | 22.42 | 0.49 | 1.82 | 0.48 | 1.22 | 2.00 | 0.80 | 1.56 | 0.29 | 0.00 | 0.00 | 0.00 |
| 36 | 2 | 2 | 4 | 8208 | 414    | 6C  | 0.78 | 0.13 | 4.19 | 13.87 | 7.41 | 44.42 | 22.04 | 0.24 | 0.89 | 0.40 | 1.26 | 2.14 | 0.88 | 1.07 | 0.28 | 0.00 | 0.00 | 0.00 |
| 37 | 2 | 2 | 4 | 8208 | 416    | 7A  | 0.73 | 0.12 | 3.68 | 12.96 | 5.49 | 45.90 | 23.40 | 0.30 | 1.10 | 0.44 | 1.47 | 2.27 | 0.94 | 0.92 | 0.30 | 0.00 | 0.00 | 0.00 |
| 38 | 2 | 2 | 4 | 8208 | 418    | 7C  | 1.26 | 0.26 | 3.66 | 10.70 | 5.61 | 40.24 | 26.78 | 0.83 | 1.67 | 0.49 | 2.21 | 1.10 | 1.38 | 3.06 | 0.48 | 0.00 | 0.00 | 0.00 |
| 39 | 2 | 2 | 4 | 8208 | 420    | 8A  | 0.75 | 0.08 | 3.58 | 10.80 | 9.42 | 44.33 | 18.68 | 0.00 | 0.80 | 0.90 | 2.47 | 3.46 | 0.75 | 0.76 | 0.32 | 0.91 | 0.42 | 0.00 |
| 40 | 2 | 2 | 4 | 8208 | 430    | 10B | 1.09 | 0.17 | 3.21 | 10.36 | 5.70 | 43.22 | 22.53 | 0.00 | 0.88 | 1.29 | 4.25 | 2.30 | 1.40 | 0.83 | 0.44 | 1.76 | 0.00 | 0.00 |
| 41 | 2 | 2 | 4 | 8208 | 413    | 6B  | 0.78 | 0.37 | 3.97 | 14.28 | ND   | 38.32 | 22.10 | 0.09 | 0.87 | 0.46 | 1.52 | 3.60 | 0.99 | 0.12 | 0.42 | 1.12 | 0.00 | 0.00 |
| 42 | 2 | 2 | 4 | 8208 | 417    | 7B  | 0.97 | 0.40 | 3.69 | 12.75 | ND   | 40.74 | 25.47 | 0.18 | 0.91 | 0.53 | 2.33 | 2.78 | 1.64 | 0.19 | 0.51 | 1.61 | 0.00 | 0.00 |
| 43 | 2 | 2 | 4 | 8208 | 421    | 8B  | 0.88 | 0.45 | 4.80 | 12.49 | ND   | 41.19 | 22.96 | 0.14 | 0.88 | 0.50 | 1.46 | 1.41 | 0.90 | 0.54 | 0.47 | 1.03 | 0.39 | 0.00 |
| 44 | 2 | 2 | 4 | 8208 | 425-9A | W   | 1.04 | 0.44 | 4.60 | 13.53 | ND   | 41.67 | 24.18 | 0.17 | 0.95 | 0.57 | 2.14 | 1.57 | 1.10 | 0.58 | 0.53 | 1.45 | 0.19 | 0.00 |
| 45 | 2 | 2 | 4 | 8208 | 429    | 10A | 0.92 | 0.35 | 3.48 | 11.79 | ND   | 42.47 | 26.18 | 0.08 | 0.74 | 0.44 | 2.29 | 2.71 | 1.11 | 0.31 | 0.00 | 1.46 | 0.00 | 0.00 |
| 46 | 3 | 4 | 6 | 8008 | 308    | D   | 1.04 | 0.38 | 6.23 | 10.43 | 6.73 | 46.63 | 14.19 | 0.60 | 1.31 | 0.07 | 3.27 | 5.45 | 1.31 | 1.09 | 0.20 | 1.03 | ND   | 0.05 |
| 47 | 3 | 4 | 6 | 8009 | 310    | D   | 1.06 | 0.38 | 5.72 | 10.19 | 6.72 | 47.14 | 17.52 | 1.26 | 1.54 | 0.07 | 3.01 | 2.54 | 0.40 | 1.29 | 0.19 | 0.96 | ND   | 0.00 |
| 48 | 4 | 5 | 7 | 7913 | 165    | D   | 1.06 | 0.34 | 2.24 | 11.02 | 2.04 | 49.96 | 22.71 | 0.06 | 0.89 | 0.07 | 4.26 | 2.99 | 0.26 | 0.21 | 0.10 | 1.76 | ND   | 0.04 |
| 49 | 4 | 5 | 7 | 7913 | 196    | D   | 1.18 | 0.38 | 2.39 | 10.90 | 1.71 | 49.34 | 23.03 | 0.05 | 0.95 | 0.07 | 4.13 | 2.83 | 0.29 | 0.40 | 0.11 | 1.78 | ND   | 0.44 |
| 50 | 4 | 5 | 7 | 7914 | 199    | B   | 0.79 | 0.37 | 0.88 | 6.20  | 2.73 | 42.20 | 36.63 | 0.08 | 0.61 | 0.35 | 4.06 | 2.80 | 0.30 | TR   | 0.34 | 1.66 | TR   | 0.00 |
| 51 | 4 | 5 | 7 | 7914 | 160    | D   | 1.14 | 0.45 | 2.92 | 11.53 | 4.16 | 49.52 | 20.08 | 0.03 | 0.93 | 0.07 | 3.96 | 2.29 | 0.26 | 0.85 | 0.09 | 1.72 | ND   | 0.01 |
| 52 | 4 | 5 | 7 | 7915 | 180    | B   | 1.11 | 0.59 | 2.85 | 9.71  | 3.10 | 41.56 | 30.20 | 0.06 | 0.83 | 0.49 | 3.48 | 3.52 | 0.33 | 0.00 | 0.36 | 1.77 | 0.00 | 0.00 |
| 53 | 4 | 5 | 7 | 7915 | 250    | B   | 0.49 | 0.36 | 0.62 | 10.13 | ND   | 51.15 | 26.68 | 0.06 | 0.01 | 0.00 | 1.50 | 2.05 | 0.00 | 0.58 | 0.52 | 3.34 | 0.00 | 0.00 |
| 54 | 4 | 5 | 7 | 7916 | 163    | D   | 0.91 | 0.40 | 2.35 | 11.50 | 1.75 | 51.07 | 21.43 | 0.05 | 0.83 | 0.05 | 4.38 | 2.76 | 0.23 | 0.43 | 0.08 | 1.78 | ND   | 0.00 |
| 55 | 4 | 5 | 7 | 8010 | 300    | D   | 0.86 | 0.29 | 2.05 | 10.98 | 0.42 | 52.40 | 22.69 | 0.02 | 0.77 | 0.03 | 4.71 | 2.23 | 0.23 | 0.37 | 0.15 | 1.78 | ND   | 0.00 |
| 56 | 4 | 5 | 7 | 8028 | 329    | D   | 1.03 | 0.45 | 2.68 | 12.45 | 1.29 | 51.02 | 20.16 | 0.14 | 0.87 | 0.04 | 4.80 | 2.07 | 0.24 | 0.83 | 0.11 | 1.83 | ND   | 0.01 |
| 57 | 4 | 6 | 8 | 7934 | 261    | B   | 0.71 | 0.54 | 2.34 | 8.76  | 2.44 | 42.84 | 32.08 | 0.00 | 0.05 | 0.43 | 1.76 | 0.56 | 0.01 | 5.76 | 0.96 | 0.78 | 0.00 | 0.00 |
| 58 | 4 | 6 | 8 | 7934 | 263    | D   | 1.05 | 0.51 | 2.84 | 9.82  | 2.24 | 49.49 | 25.74 | 0.02 | 0.73 | 0.16 | 2.02 | 4.13 | 0.31 | 0.17 | 0.13 | 0.59 | ND   | 0.05 |
| 59 | 4 | 6 | 8 | 7936 | 277    | D   | 1.03 | 0.36 | 2.63 | 11.19 | 2.82 | 51.42 | 22.07 | 0.11 | 0.66 | 0.17 | 2.13 | 3.85 | 0.19 | 0.23 | 0.13 | 0.58 | ND   | 0.42 |

*Set 2 samples.* Set 2 supplied data on 27 samples from 10 species ("ANAL" = D and Group is blank in Table 3). The most divergent amino acid values are those for phenylalanine in OBS 2 and 29, being 9.85 and 9.62 residues per 100 residues. These unusually high and very similar values came from two species collected over one year apart. The high levels of phenylalanine appear to be associated with low levels of glycine, but the two cannot be confused in the analytical procedure. Nevertheless, these results appeared to be so incongruous that every effort was made to locate some extraneous source of error, but none was found. On the basis of levels of phenylalanine alone, statistical analysis placed these two samples into a statistical population quite separate from all of the other samples in Table 3. Accordingly, the data from them have not been included in the statistical analyses made on the remaining samples.

*Set 3 samples.* In order to determine whether there are timewise trends in chemical composition as MaAS is generated by spiders, the set 3 samples were secured as groups of chronologically (A to C) related subsamples, each group (1 to 10) representing a single, continuous, forcible silking lasting up to 20 minutes. The inability of the Waters instrument to determine proline complicated, but did not prevent the analysis of the results. In Table 3, it is seen that in each of groups 1, 3, 6 and 7 the Durrum results are far from being identical for subsamples A and C. If there are timewise trends peculiar to a single spider, the increase or decrease or lack of change in the level of each amino acid for subsamples A and C, should be the same or at least in the same direction, in duplicate silkings. Group 1 (OBS 10 and 11) and group 3 (OBS 13 and 14) are duplicates from spider 8205 (*N. hentzii*). The six amino acids which compose about 91% of the total residues provide the following directions of change. Gly increases between OBS 10 and 11 but decreases between OBS 13 and 14. Similarly, Ala increases vs. increases; Glu is essentially unchanged vs. decreases; Pro decreases vs. increases; Ser increases vs. decreases; Val decreases vs. increases. For spider 8208 (*A. aurantia*) group 6 (OBS 35 and 36) vs. group 7 (OBS 37 and 38); Glu increases vs. decreases; Ala essentially unchanged vs. decreases; Gly increases vs. decreases; Pro decreases vs. virtually unchanged; Ser increases vs. identical; Val decreases vs. increases.

From these observations it is reasonable to conclude that there are no systematic trends in the changing chemical compositions during the progression of forcible silkings of individual spiders. This conclusion is reinforced when proline interpolations are put into the Waters data and all of the Set 3 results are submitted to statistical trend analyses.

There is a second method which has the added advantages of reducing theoretical instrument-operator errors and allows the usage of Waters data without taking proline into account. It makes use of dimensionless pair ratios of amino acids from each analysis, of which Gly/Ala, Glu/Pro and Ser/Tyr are useful examples, particularly Gly/Ala, since for the Waters analyses both came from the same aliquot of the primary hydrolysate. The Glu/Pro pair does not exist from the Waters analyses but Glu may be paired with any other amino acid. When these ratios were submitted to statistical analysis, no uniform directional timewise trends were found in compositions of MaAS taken during single forcible silkings.

**Taxonomy.**—In Table 3, each of 57 usable (omitting OBS 2 and 29) analytical results report upon a maximum of 18 amino acids from 26 spiders, 4 subfamilies,

8 genera, 11 species and 3 instrument-operator combinations, thereby providing an extremely large number of potential sets for statistical analyses. Each taxon-analysis combination ( $n = 54$ ) was taken as a statistical set and submitted to the Duncan Multiple Range Test, programmed for an IBM® 3081 computer at the Triangle Universities Computer Center by SAS®. The results are given in Table 4. The Duncan Test has been described in standard textbooks (example, Steel and Torrie), its computer usage by SAS® (1982) and is summarized (Robert J. Monroe, per. comm. 1986) as follows:

“The Duncan Multiple Range Procedure was designed to separate a heterogeneous group of mean values into subsets each of which is judged homogeneous in the statistical sense of the word. In its usual form, the procedure identifies each homogeneous subset by a different letter (A, B, C . . .). Since the means are ranked from high to low the letters form a progression from A, B, C . . . depending on the number of different subsets. Hence when a given mean value may belong to two or more subsets, more than one letter may appear beside the mean indicating this fact. In some few instances there may be a clear delineation of subsets but the usual case almost always has some overlapping subsets; thus the source of ambiguity in the interpretation. A common logical error is to equate ‘non-significance’ (using a test level of Prob. = 0.05) to ‘equality’. This reasoning process then always leads to a violation of one of the canons “Things equal to the same thing are equal to each other”. A more reasoned use is to impute to ‘non-significance’ the inability to discriminate among means without concluding equality among them. In this case no logical contradictions occur”.

In programming the Duncan procedure, one level of possible error is used for species, two for genera and three for subfamily. For inclusion in Table 4 the more (genera) or most (subfamily) conservative result was selected on the basis of having the greater (-est) mean square error. In examining the letter designations in Table 4, it must be kept in mind that only those within a set may be intercompared.

The only amino acids which help distinguish subfamilies are Pro and Cys. Little weight can be given, however, to the Cys differences, because cysteine is present in very small amounts (Table 3), and  $n$  is only 2 for Metinae, the subfamily with unique Cys values. On the other hand, Nephilinae (and its genera and species) is distinct from the other taxa on the basis of Pro which is present in large amounts.

The forcible silkings that provided 59 analyses of MaAS, gave only enough MiAS for the 11 seen in Table 5. These limited results were submitted to statistical analyses, from which it can only be said that these carry the hint that there is less variability of amino acid composition for MiAS, both within species and between species, than for corresponding MaAS. It should be kept in mind, however, that one uncertainty found for MaAS does not exist with MiAS, namely, there is no present evidence that MiAS may exist as two-component systems.

**Macromolecular chemistry.**—It is generally accepted that in the fibroin of the silk of *Bombyx mori* Linnaeus there is a sequence of (Gly-X-Gly-X-Gly-X)<sub>n</sub>, where X may be either Ala or Ser, these last being in the ratio of two to one, respectively. Although investigators do not agree upon the exact composition of silk from *B. mori* (Lucas, et al. 1960; Iizuka 1970; Komatzu 1979), the analyses are close to the ratios, Gly = 3; Ala = 2; Ser = 1). As Dickerson and Geis (1969) point out, this allows the glycine side chains to “nestle quite efficiently” opposite to the larger alanine or serine side chains in the antiparallel-chain pleated sheet model of Pauling and Corey (1953). This applies only to the crystalline domains

Table 4.—Duncan Multiple Range Test on major ampullate silk analyses, by taxa: \*Number of results secured. \*\*Maximum samples available.

| Taxa           | n* | ASP | THR | SER | GLU | PRO | GLY | ALA  | CYS | VAL | ILE | LEU | TYR | PHE | HIS | LYS | ARG | TRY | MET |
|----------------|----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                |    | 57  | 57  | 57  | 57  | 46  | 57  | 57   | 53  | 57  | 56  | 57  | 56  | 54  | 50  | 57  | 57  | 2   | 16  |
| <b>SF</b>      |    |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |
| 1              | 20 | A   | A   | A   | A   | A   | A   | A    | B   | A   | A   | A   | A   | A   | A   | A   | A   | A   | A   |
| 2              | 23 | A   | A   | A   | A   | A   | A   | A    | B   | A   | A   | A   | A   | A   | A   | A   | A   | A   | A   |
| 3              | 2  | A   | A   | A   | A   | A   | A   | A    | A   | A   | A   | A   | A   | A   | A   | A   | A   | -   | A   |
| 4              | 12 | A   | A   | A   | A   | B   | A   | A    | B   | A   | A   | A   | A   | A   | A   | A   | A   | -   | A   |
| <b>Genera</b>  |    |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |
| 1              | 3  | A   | BC  | A   | A   | AB  | AB  | BC   | B   | AB  | A   | B   | A   | A   | A   | A   | B   | -   | A   |
| 2              | 23 | A   | BC  | A   | A   | BC  | B   | ABC  | B   | AB  | A   | B   | A   | A   | A   | A   | B   | A   | A   |
| 3              | 3  | A   | C   | A   | A   | AB  | A   | C    | B   | AB  | A   | B   | A   | A   | A   | A   | B   | -   | A   |
| 4              | 2  | A   | BC  | A   | A   | C   | AB  | BC   | A   | AB  | A   | A   | A   | A   | A   | A   | B   | -   | A   |
| 5              | 9  | A   | BC  | A   | A   | D   | AB  | AB   | B   | AB  | A   | A   | A   | A   | A   | A   | A   | -   | A   |
| 6              | 3  | A   | B   | A   | A   | D   | AB  | A    | B   | B   | A   | B   | A   | A   | A   | A   | B   | -   | A   |
| 7              | 1  | A   | C   | A   | A   | A   | B   | ABC  | B   | AB  | A   | B   | A   | A   | A   | A   | B   | -   | A   |
| 8              | 13 | A   | A   | A   | A   | ABC | B   | ABC  | B   | A   | A   | B   | A   | A   | A   | A   | B   | A   | A   |
| <b>Species</b> |    |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |
| 1              | 2  | A   | AB  | ABC | BC  | AB  | AB  | BCD  | B   | ABC | AB  | BC  | A   | B   | A   | A   | B   | -   | A   |
| 2              | 1  | A   | B   | BCD | A   | A   | AB  | D    | B   | ABC | ABC | C   | A   | B   | A   | A   | B   | -   | A   |
| 3              | 4  | A   | B   | ABC | BC  | AB  | AB  | ABCD | B   | BC  | ABC | BC  | A   | AB  | A   | A   | AB  | -   | A   |
| 4              | 19 | A   | B   | CDE | B   | AB  | B   | AB   | B   | ABC | AB  | BC  | A   | A   | A   | A   | AB  | A   | A   |
| 5              | 2  | A   | B   | A   | C   | A   | A   | D    | B   | ABC | ABC | C   | A   | B   | A   | A   | B   | -   | A   |
| 6              | 2  | A   | B   | AB  | BC  | B   | AB  | BCD  | A   | AB  | C   | AB  | A   | AB  | A   | A   | AB  | -   | A   |
| 7              | 9  | A   | AB  | E   | BC  | C   | AB  | A    | B   | BC  | BC  | A   | A   | B   | A   | A   | A   | -   | A   |
| 8              | 3  | A   | AB  | DE  | BC  | C   | AB  | A    | B   | C   | ABC | BC  | A   | B   | A   | A   | B   | -   | A   |
| 9              | 1  | A   | B   | A   | BC  | A   | B   | ABCD | B   | ABC | A   | C   | A   | B   | A   | A   | B   | -   | A   |
| 10             | 1  | A   | B   | ABC | BC  | A   | AB  | CD   | B   | BC  | AB  | C   | A   | B   | A   | A   | B   | -   | A   |
| 11             | 13 | A   | A   | ABC | BC  | AB  | B   | ABC  | B   | A   | AB  | C   | A   | AB  | A   | A   | B   | A   | A   |

of *B. mori*, which constitute about 37% to 47% of the total, as defined by x-ray diffraction analysis (Iizuka 1965).

The considerable differences in amino acid compositions of MaAS and MiAS from *Araneus diadematus* and *Argiope aurantia*, respectively, have already been illustrated by Andersen (1970) and Tillinghast (1984), respectively. The former has underscored this by comparing (his Table 2) the residues with short side chains in MaAS and MiAS, being 62.25 and 84.60. In the present study the corresponding figures are 70.47 ( $n = 57$ ) and 83.45 ( $n = 11$ ). It must be mentioned, however, that the C.V.s of the mean values for MaAS are quite large, being Gly = 8.89%, Ala = 20.8%, Ser = 37.4%, and MiAS, the same amino acids, C.V.s are 13.1%, 9.14%, 25.2%. As an extension of this approach, *t*-tests were made to compare the mean values of corresponding amino acids in MaAS (Table 3) vs. those for MiAS (Table 5). It was found that significant or highly significant differences existed for all amino acid residues except for Ser and four of those present in very small amounts, Cys, Met, Phe, and His.

It is evident that when compared with MaAS, the higher levels of short side chain amino acids make it more possible for MiAS to approach the conformational structure of the anti-parallel chain pleated sheet model. To what degree this theoretical condition is attained remains unknown, since the crystalline-amorphous ratios of these two silks have yet to be determined. It would be expected that the relative amounts of amorphous domains, and their chemical and conformational aspects, would be determinative, relative to physico-chemical properties. Some hints relative to this may be revealed by the physical properties of the two types of fibers, investigated many years ago in the pioneering paper by Lucas, et al. (1955). Thus it is not surprising that the 'Hookean' section of the force-elongation curves of MiAS reach higher levels of stress than do MaAS, before inflection (Work 1977, figs. 1 and 2, and *ibid.*, Table 1, section C) and that MiAS possess higher birefringences than MaAS. Levels of this property are considered to be functions of the vectorial degree of alignment of molecular segments parallel to the axis of the fibers, as induced by amounts of 'drawing'. The chemical composition data which suggests that MiAS has greater structural order than MaAS is consistent with the higher birefringences of MiAS.

Tillinghast and Christenson (1984) have already raised the question of the effect of proline on supercontraction. Some light can now be cast on the subject. Because nothing is known regarding sequence of amino acids in either MaAS or MiAS, only average values for amino acids with large side groups may be considered. The Pro in MaAS from the combined subfamilies Araneinae and Argiopinae ( $n = 33$ ) has a mean value of 9.14 (C.V. = 23.1%); for Nephilinae ( $n = 11$ ) mean = 2.25 (C.V. = 40.4%) (Metinae,  $n = 2$ , will be disregarded). For all MiAS ( $n = 11$ ) mean = 0.46 (C.V. = 74%). Thus, the potentiality exists, on the average, that a proline may occur every 11 amino acid units for the first two subfamilies, every 44 for Nephilinae, and only every 220 for MiAS. Glutamic acid is another amino acid with a large side group. When similar calculations are made on its presence in MaAS and MiAS, it is found that based on average contents, it may occur about every nine and 50 units in these two polypeptides, respectively. Work (1981b, Table 1), has shown that combined Araneinae and Argiopinae ( $n = 180$ ) mean supercontraction ratio = 0.546; for *Nephila clavipes* ( $n = 29$ ) mean ratio = 0.639; and for *Nephilengys cruentata*, ( $n = 22$ ), mean ratio = 0.817. The contraction ratio of MiAS was 0.956 ( $n = 35$ ). Thus, there is an

Table 5.—Analyses of amino acid composition of minor ampullate silk samples: ND = Not determined. Column headings and code numbering are explained in the Results section of the text.

|   | S  | S |   | ASP | THR  | SER  | GLU  | PRO  | GLY  | ALA   | CYS   | VAL  | ILE  | LEU  | TYR  | PHE  | HIS  | LYS  | ARG  | TRY  | MET  |      |
|---|----|---|---|-----|------|------|------|------|------|-------|-------|------|------|------|------|------|------|------|------|------|------|------|
| O | 1  | 1 | 1 | 1   | 1.71 | 1.65 | 4.15 | 1.98 | 0.38 | 47.46 | 30.26 | 0.39 | 1.65 | 0.33 | 1.35 | 5.63 | 0.74 | 0.77 | 0.11 | 1.44 | ND   | 0.00 |
| B | 1  | 1 | 1 | 1   | 1.87 | 1.48 | 4.60 | 1.89 | 0.58 | 51.09 | 29.56 | 0.44 | 1.38 | 0.27 | 0.60 | 2.35 | 0.59 | 1.53 | 0.20 | 1.57 | ND   | 0.00 |
| S | 3  | 1 | 3 | 5   | 0.95 | 1.83 | 4.02 | 0.98 | 0.39 | 54.70 | 28.16 | 0.02 | 0.96 | 1.02 | 1.07 | 4.49 | 0.24 | 0.24 | 0.06 | 0.73 | ND   | 0.15 |
| F | 4  | 1 | 3 | 5   | 0.98 | 1.68 | 3.75 | 0.96 | 0.34 | 54.07 | 28.68 | 0.04 | 1.14 | 0.97 | 1.01 | 4.98 | 0.41 | 0.18 | 0.07 | 0.71 | ND   | 0.02 |
| G | 5  | 1 | 3 | 10  | 1.00 | 1.52 | 3.62 | 1.01 | 0.20 | 55.75 | 28.37 | 0.03 | 0.92 | 0.78 | 1.08 | 4.36 | 0.25 | 0.25 | 0.04 | 0.77 | ND   | 0.06 |
| P | 6  | 1 | 8 | 11  | 1.19 | 0.72 | 2.29 | 0.75 | 0.00 | 58.33 | 29.51 | 0.25 | 1.14 | 0.91 | 0.62 | 3.36 | 0.17 | 0.68 | 0.08 | 0.00 | ND   | 0.00 |
| R | 7  | 2 | 2 | 3   | 1.85 | 1.70 | 5.27 | 2.99 | 1.12 | 41.98 | 31.88 | 0.12 | 1.38 | 1.24 | 1.77 | 4.68 | 0.77 | 0.00 | 1.08 | 2.17 | 0.00 | 0.00 |
| E |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
| P |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
| L |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
| A |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
| G |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
| R |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
| A |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
| N |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
| A |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
|   | 8  | 2 | 2 | 4   | 1.82 | 1.87 | 4.52 | 1.46 | 0.00 | 40.95 | 35.49 | 0.08 | 1.69 | 1.15 | 1.93 | 4.72 | 0.65 | 0.00 | 0.71 | 2.22 | 0.00 | 0.00 |
|   |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
|   |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
|   |    |   |   |     |      |      |      |      |      |       |       |      |      |      |      |      |      |      |      |      |      |      |
|   | 9  | 4 | 5 | 7   | 2.32 | 2.12 | 4.46 | 3.60 | 0.62 | 45.86 | 27.63 | 0.13 | 2.84 | 1.34 | 1.78 | 3.77 | 0.58 | 0.80 | 0.29 | 1.82 | ND   | 0.04 |
|   | 10 | 4 | 6 | 8   | 1.50 | 1.32 | 2.86 | 2.93 | 0.56 | 39.95 | 35.21 | 0.01 | 1.62 | 0.71 | 1.00 | 5.04 | 0.65 | 2.67 | 1.81 | 1.61 | ND   | 0.44 |
|   | 11 | 4 | 6 | 8   | 1.23 | 0.88 | 2.35 | 3.31 | 0.85 | 52.81 | 28.34 | 0.03 | 1.39 | 0.82 | 0.99 | 4.59 | 0.42 | 0.42 | 0.08 | 1.49 | ND   | 0.00 |

inverse relationship between the presence of the large side groups of proline and glutamic acid in the polypeptide molecules and the degree of their axial stability, when wetted in an unrestrained state.

The physical characteristics of MaAS, strength, extensibility, moduli, and viscoelastic behavior, regardless of source of these fibers, are very similar (Work 1976, 1977, 1981b, 1985). Because the knowledge of these is infinitesimally small as compared with the published literature on man-made fibers, it is appropriate to consider the latter as the basis of speculation about the former. Among man-made fibers, very small differences in chemical composition tend to produce considerable dissimilarities in physico-chemical properties, as is well known to polymer chemists who have attempted to duplicate the properties of commercially acceptable man-made fibers without infringing patents. In view of this it may be speculated that perhaps the core dominates physical properties, even as it alone supercontracts. It is of interest to note that MiAS and the skin of MaAS neither supercontract nor possess a known function in the orb web. One can only conjecture concerning all of these phenomena, and suggest that their study, especially their individual chemical compositions and amino acid sequencing, offer a challenge for future study.

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